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(54) Title: BIOLUMINESCENCE REGENERATIVE CYCLE (BRC) FOR NUCLEIC ACID QUANTIFICATION

(57) **Abstract:** The present invention concerns methods of quantifying nucleic acids using a bioluminescence regenerative cycle (BRC). In BRC, steady state levels of bioluminescence result from processes that produce pyrophosphate. Pyrophosphate reacts with APS in the presence of ATP sulfurylase to produce ATP. The ATP reacts with luciferin in a luciferase-catalyzed reaction, producing light and regenerating pyrophosphate. The pyrophosphate is recycled to produce ATP and the regenerative cycle continues. Because the kinetic properties of ATP sulfurylase are much faster than luciferase, a steady state results wherein concentrations of ATP and pyrophosphate and the rate of light production remain relatively constant. Photons are counted over a time interval to determine the number of target molecules present in the initial sample. The BRC process has a controllable dynamic range up to seven orders of magnitude and is sensitive enough to detect a few thousand molecules of target nucleic acid.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/20690

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12Q 1/68; C12P 19/34; C07H 21/04  
 US CL : 435/6, 91.2; 536/24.33

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| Y          | US 6,274,320 B1 (ROTHBERG et al.) 14 August 2001 (14.08.2001), entire document, especially column 16, lines 7-67, column 17, lines 1-61. | 1-35                  |
| Y          | US 6,270,973 B1 (LEWIS et al) 07 August 2001 (07.08.2001) entire document, especially column 46, lines 19-67, column 47, lines 1-53.     | 1-35                  |
| Y,E        | US 6,534,269 B2 (LIU et al) 18 March 2003 (18.03.2003), entire document, especially column 3, lines 35-67, column 4, lines 1-48.         | 1-35                  |
| Y          | US 6,255,083 B1 (WILLIAMS) 03 July 2001 (03.07.2001), entire document.   | 1-35                  |

 Further documents are listed in the continuation of Box C. See patent family annex.

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**Continuation of B. FIELDS SEARCHED Item 3:**  
Biosis, Embase, Medline, Lifesci, Caplus, East databases  
search terms: multiple targets, pyrophosphate, sequencing, rolling circle